ACTA BIOLOGICA CRACOVIENSIA Series Botanica 56/1: 28–41, 2014
DOI: 10.2478/abcsb-2014-0002

CELL PATTERN AND ULTRASCULPTURE OF BULB TUNICS OF SELECTED *ALLIUM* SPECIES (AMARYLLIDACEAE), AND THEIR DIAGNOSTIC VALUE

KAJA ROLA*

Department of Plant Taxonomy, Phytogeography & Herbarium, Institute of Botany, Jagiellonian University, Kopernika 27, 31-501 Cracow, Poland

Received October 10, 2013; revision accepted December 12, 2013

Cell pattern and ultrasculpture were examined by light and scanning electron microscopy in bulb tunics of 46 *Allium* species to determine the diagnostic value of micromorphological characters. The study examined the diversity of these characters, evaluated their usefulness at different taxonomic levels (species, section, subgenus), and considered the results in relation to the recent intrageneric classification of the genus. Detailed characteristics are provided for the investigated species, and taxa showing the presence of calcium oxalate crystals in bulb tunic cells are indicated. The results suggest that several bulb tunic characters are of taxonomic significance in *Allium* as their variation between specimens of the same species was negligible; they can be useful elements of species descriptions and determination keys. *Allium* subgenus *Allium* shows considerable variation of bulb tunic ultrasculpture and hexagonal or elongated cell patterns. Differences in ultrasculpture are sufficient to distinguish species within the *Amerallium* subgenus. Three subgenera (*Anguinum*, *Butomissa*, *Reticulatobulbosa*) are characterized by fibrous tunics with reticulate ultrasculpture. Rectangular to elliptic cells with thick walls, giving the bulb tunic an almost perforated structure, are characteristic for *Allium* subgenus *Cepa*. No specific pattern was found for *Allium* subgenus *Melanocrommyum* and *Polyprason*. The only representative of subgenus *Microscordum* (*Allium monanthum*) showed distinct herringbone ultrasculpture. The bulbs of *Allium* subgenus *Rhizirideum* representatives can be distinguished by their linear ultrasculpture following the long axis of the elongated cells. *Allium* bulb tunic ultrasculpture and cell pattern show some degree of variability. These characters are of potential use in taxonomic delimitation, species determination and further study of the relationships between species, particularly in members of subgenus *Amerallium*.

Key words: *Allium*, bulb tunic, calcium oxalate crystals, micromorphology, SEM, taxonomy, ultrasculpture.

INTRODUCTION

The genus *Allium* L. includes over 800 species, 15 subgenera and more than 70 sections (Friesen et al., 2006; Fritsch et al., 2010) of herbaceous perennial plants with natural distributions in the Northern Hemisphere (Stearn, 1978, 1980; Friesen, 1988; Friesen et al., 2006; Fritsch and Abbasi, 2009). The genus *Allium* was previously classified as a member of the Lilliaceae family and then Alliaceae, subfamily Allioideae Herb. (Fay and Chase, 1996). Recently *Allium* has been placed within Amaryllidaceae (APG III, 2009). The genus is characterized by the presence of bulbs enclosed in membranous (sometimes becoming fibrous) tunics, free or almost-free tepals and often a subgynobasic style (Friesen et al., 2006). The diversity of *Allium* is greatest in Southwest and Central Asia and the Mediterranean region, and there is a second smaller center of diversity in North America (McNeal, 1992; Friesen et al., 2006; Nguyen et al., 2008).

Several criteria have been used in taxonomic classification of *Allium*. Some characters have proved useful at subgeneric and sectional levels, including the structure and shape of rhizome and bulb, and anatomical features of root, leaf, scape, ovary, septal nectaries and seed (De Wilde-Duyfjes, 1976; Fritsch, 1992, 1993; Kruse, 1992; McNeal, 1992; Friesen et al., 2006; Gurushidze et al., 2008; Nguyen et al., 2008; Choi and Cota-Sánchez, 2010; Celep et al., 2012). Many other characters are diagnostically important and commonly used in species descriptions. Scanning electron microscopy (SEM) has greatly improved the taxonomy of the genus,

*e-mail: kajaskubala@interia.pl

PL ISSN 0001-5296

facilitating studies of the ultrasculpture and ornamentation of the seed coat (Pastor, 1981; Kruse, 1984, 1992; Češmedžiev and Terzijski, 1997; Fritsch et al., 2006; Neshati and Fritsch, 2009; Celep et al., 2012; Choi et al., 2012), pollen grains (Güler and Pehlivan, 2006; Namin et al., 2009), bulb coats (De Wilde-Duyfjes, 1976; McNeal, 1982, 1992; Choi and Oh, 2011), floral structures (Choi et al., 2011) and leaf epidermal cells (Garbari et al., 1979; Krahulec, 1980; Choi et al., 2004; Choi and Oh, 2011).

The bulb plays an important role in species identification within the genus *Allium*. Bulb structure, shape and size and especially the texture of the outer bulb tunic play a great part in species diagnosis (De Wilde-Duyfjes, 1976; McNeal, 1982; Speta, 1984; Pastor and Valdés, 1986; McNeal and Jacobsen, 2002). Bulb tunic ornamentation has been used to elucidate the relationships among North American species (McNeal, 1992) and East Asian species (Choi and Oh, 2011). The bulb tunic develops through differential lignification of the inner and radial cell walls of the adaxial epidermis of the uppermost foliage leaf (McNeal and Ownbey, 1973). Depending on the lignification processes, two bulb coat types have been distinguished (fibrous-reticulate, smooth nonreticulate), and the usefulness of tunic cell pattern and shape in distinguishing particular species has been noted (McNeal, 1992). Nevertheless, the use of bulb tunic ornamentation and cell pattern for identifying *Allium* species is rarely considered, especially in reference to comprehensive classification of the genus.

What is the relationship between micromorphology and the molecular data used to reconstruct *Allium* phylogeny? The bulb tunic ornamentation of some species has not been investigated previously. In this work I examine these microstructures in order to assess their usefulness for the classification of *Allium* proposed by Friesen et al. (2006), and specifically, to study the diversity of bulb tunic cell pattern and ultrasculpture in selected *Allium* species, to assess the diagnostic value of these characters at different taxonomic levels (species, section, subgenus), and to consider the results in relation to the recent intrageneric classification.

MATERIALS AND METHODS

The ultrasculpture of the bulb tunics of 46 selected *Allium* species representing 10 subgenera and 21 sections was examined by scanning electron microscopy (SEM) (JEOL JSM-5410 and NORAN Vantage) at 15 kV accelerating voltage. The samples were sputter-coated with gold (for details see Rola, 2012). The observations used 92 herbarium specimens (two of each species when available, two samples from one specimen when not). The specimens are housed in KRA (Jagiellonian University, Cracow). When possible the specimens were selected from different parts of the species' distribution range. Next, epidermal peels of the inner surface of the bulb tunic of each species (except for species with typical reticulate or crustaceous outer tunics) were examined by light microscopy (LM) to observe their cell shape patterns. Table 1 lists the examined species together with herbarium collection data (when labels were legible). The terminology describing bulb tunic ultrasculpture follows De Wilde-Duyfjes (1976) and McNeal (1992).

RESULTS AND DISCUSSION

The diversity of bulb tunic texture, ultrasculpture and cell patterns of the studied *Allium* species are presented in Figures 1–7 and Table 1. In most cases the texture of bulb tunics was membranous or papery (Tab. 1). In some it was fibrous, and the fibers were more or less parallel (e.g., *A. moschatum*, Fig. 4a) or formed a reticulate structure (taxa of 3 subgenera: *Reticulatobulbosa*, *Butomissa* and *Anguinum*, Figs. 2h,i, 3a, 4b–g). In some species, mainly from subgenus *Amerallium*, the structure of outer tunics was chartaceous or crustaceous with distinctive ultrasculpture visible under SEM (e.g., Fig. 2d–f). These characters of the outer bulb tunic apparently are of high diagnostic value. I determined some species-specific and subgenera-specific characters for which variation between specimens representing the same species is negligible; specimens from different geographical areas (Tab. 1) showed uniform outer bulb tunic ultrasculpture and cell pattern. These characters are stable and therefore can be successfully used in species descriptions and determination keys.

Some taxa had numerous calcium oxalate crystals visible by LM, or in the form of verrucae under SEM (Tab. 1). The crystals are actually intracellular, embedded in the cell mesophyll (Prychid and Rudall, 1999); that is why only their shapes were visible by SEM. This study supports Jaccard and Frey's (1928) suggestion that crystal types are useful for determining some species but not for the whole systematics of the genus. In contrast, Chartschenko (1932) distinguished 8 types and forms of calcium oxalate crystals in *Allium* and remarked that they play an important role in phylogenetic systematics. However, his classification of certain species differs from the recent intrageneric classification (Friesen et al., 2006). I found calcium oxalate crystals in the outer bulb tunics of 23 of the examined species (Tab. 1), but no specific types of oxalate crystals were characteristic of subgenera or sections.

In the present work the main differences in bulb tunic ultrasculpture and cell pattern were at species level; specific characters for only a few subgenera were identified. Convergent evolution can lead to sim-

TABLE 1. Main characteristics of outer bulb tunics of examined *Allium* species, with herbarium data. Subgenera and sections follow Friesen et al. (2006) and Nguyen et al. (2008)

Species	Subgenus/ section	Character of outer bulb tunic	Ultrasculpture of outer bulb tunic	Cell pattern of inner surface of outer bulb tunic	Herbarium data
Allium acutiflorum Loisel.	*Allium/Allium*	membranous to coriaceous	striate	hexagonal cells with calcium oxalate crystals	France: Montagne de la Gardiole near Mireval (KRA 0284803)
Allium ampeloprasum L.	*Allium/Allium*	papery, slightly fibrous	smooth	elongated spindly cells with calcium oxalate crystals	Croatia: Vodice (KRA 0307370) Italy: Sardini, Capo Caccia (KRA 0323544) Italy: Ligurian Alps, Madonna di Palara (KRA 0326740)
Allium atroviolaceum Boiss.	*Allium/Allium*	membranous to fibrous	smooth	transversely elongated cells with calcium oxalate crystals	China: Tian Shan (KRA 0205190)
Allium polyanthum Schult. & Schult.f.	*Allium/Allium*	membranous	smooth to slightly striate	rectangular/hexagonal transversely elongated cells with calcium oxalate crystals	KRA 0205452
Allium scorodoprasum subsp. *rotundum* (L.) Stearn	*Allium/Allium*	coriaceous	pattern of hexagonal cells	hexagonal cells with calcium oxalate crystals	Poland: Niecka Nidziańska basin, Busko Zdrój (KRA 0358174) Poland: Płaskowyż Proszowicki plateau, Piotrowice Wielkie (KRA 0304490) Croatia: between Vodice and Šibenik, along Krk river (KRA 0307368)
Allium scorodoprasum L. subsp. *scorodoprasum*	*Allium/Allium*	membranous to subcoriaceous	striate	hexagonal cells and calcium oxalate crystals	Poland: Kotlina Sandomierska valley, Przychojec vill. (KRA 0361634)
Allium sphaerocephalon L.	*Allium/Allium*	membranous or coriaceous	pattern of hexagonal cells	hexagonal cells and calcium oxalate crystals	Austria: Baden, Kalvarienberg Mt. (KRA 205604) Austria: Burgenland, Hackerberg (KRA 0287168) Italy: Ligurian Alps, Pigna vill. (KRA 0326747)
Allium vineale L.	*Allium/Allium*	papyraceous	striate with verrucose ribs	elongated cells	Poland: Beskid Makowski Mts, Plebańska Góra Mt. near Myślenice (KRA 0257912) Poland: Wyżyna Kielecka upland, Eustachów vill. (KRA 0273547)
Allium carinatum L.	*Allium/ Codonoprasum*	membranous to papery	pattern of hexagonal cells	hexagonal cells with calcium oxalate crystals	Slovenia: Ilirska Bistrica near Snežnik Mt. (KRA 0307361) Czech Republic: Bile Karpaty Mts, Nová Lhota vill. (KRA 205290) Italy: Dolomites Mts, Magasa vill. (KRA 0326743)

TABLE 1. *Cont.*

Species	Subgenus/ section	Character of outer bulb tunic	Ultrasculpture of outer bulb tunic	Cell pattern of inner surface of outer bulb tunic	Herbarium data
Allium flavum L.	*Allium/ Codonoprasum*	membranous	striate with smooth ribs	elongated cells with calcium oxalate crystals	Czech Republic: Moravia, Mohelno (KRA 0376156, KRA 0376195) Hungary: Heves (KRA 0360772)
Allium melanantherum Pančić	*Allium/ Codonoprasum*	membranous	smooth	elongated cells with calcium oxalate crystals	Bulgaria: Tintjava (KRA 118239)
Allium oleraceum L.	*Allium/ Codonoprasum*	membranous	striate with striped ribs	elongated cells with calcium oxalate crystals	Poland: Wyżyna Kielecka upland, Kolonia Kuźnia vill., (KRA 0321688) Poland: Płaskowyż Proszowicki plateau, Gruszów vill. (KRA 0306534) Bulgaria: Karlovo vill. (KRA 0239207)
Allium paniculatum L.	*Allium/ Codonoprasum*	membranous to papery	pattern of hexagonal cells	hexagonal cells with calcium oxalate crystals	Hungary: Krassó-Szörény County (KRA 060752, KRA 060754)
Allium stamineum Boiss.	*Allium/ Codonoprasum*	membranous to papery	smooth with pattern of oxalate crystals	hexagonal cells with calcium oxalate crystals	Israel: Carmel Mt. near Haifa (KRA 0205636)
Allium ursinum L.	*Amerallium/ Arctoprasum*	papery	striate with pattern of elongated deltoid cells	deltoid/rectangular elongated cells with calcium oxalate crystals	Poland: Beskid Niski Mts, Krempna vill. (KRA 364311) Poland: Pogórze Śląskie foothills, Rudzica/Landek (KRA 0293825)
Allium pendulinum Ten.	*Ameralium/ Briseis*	membranous	sinuate with curved cells	irregular cells with sinuate walls	Italy: Sicily, Madonie, Vallone Pomieri (KRA 0359983) Italy: Lucania, Pignola vill. (KRA 0205450)
Allium triquetrum L.	*Ameralium/ Briseis*	membranous	smooth	rhomboid/deltoid cells	Italy: Ostriconi (KRA 0352500) Italy: Sardinia, Giara di Gesturi plateau (KRA 0323540)
Allium chamaemoly L.	*Amerallium/ Chamaeprason*	chartaceous	faveolate with rhomboidal pattern	rhomboidal cells	Italy: Province of Genoa (KRA 0205299)
Allium moly L.	*Amerallium/ Mollium*	chartaceous	conspicuously rugose-sinuate	irregular cells with thick cell walls	KRA 0205127
Allium roseum L.	*Amerallium/ Mollium*	crustaceous	faveolate with the pattern of rectangular cells and thick walls with semilunar, tubercular appendages	pattern with specific rectangular cells	Italy: Corsica, Ostraconi (KRA 0352503) Italy: Calabria, Craco vill. (KRA 0314227, KRA 03144229)
Allium subhirsutum L.	*Amerallium/ Mollium*	membranous	smooth with pattern of irregular cells with sinuous walls	irregular cells with sinuous walls	Italy: Sardinia, Caprera (KRA 0386812) Greece: Peloponnese, Kalamaki near Mili (KRA 0326991)

TABLE 1. *Cont.*

Species	Subgenus/ section	Character of outer bulb tunic	Ultrasculpture of outer bulb tunic	Cell pattern of inner surface of outer bulb tunic	Herbarium data
Allium tricoccum Sol.	*Anguinum/ Anguinum*	fibrous	reticulate	elongated cells	KRA 0290518
Allium victorialis L.	*Anguinum/ Anguinum*	fibrous	reticulate	elongated cells	Poland: Wyżyna Kielecka upland, Lipie forestry near Starachowice (KRA 0322064) Poland: Bieszczady Mts, Szeroki Wierch Mt. (KRA 0377320)
Allium ramosum L.	*Butomissa/ Butomissa*	reticulate to subreticulate	reticulate	elongated cells	China: Shandong Province, Shilaizhen (KRA 0346761)
Allium cepa L.	*Cepa/ Cepa*	chartaceous	pattern of rectangular cells	rectangular/hexagonal cells with thick walls and calcium oxalate crystals	Poland: Wysoka Nowa vill. near Szydłowiec (KRA 0320763) Poland: Płaskowyż Proszowicki plateau, Gniazdowice vill. (KRA 0306552)
Allium fistulosum L.	*Cepa/ Cepa*	chartaceous	pattern of rectangular cells	rectangular cell with thick walls and calcium oxalate crystals	Poland: Nizina Śląska lowland, Kluczbork (KRA 0137200, KRA 030864)
Allium sibiricum L.	*Cepa/ Shoeneoprasum*	papyraceous to subcoriaceous	pattern of rectangular cells	rectangular cell with thick walls and calcium oxalate crystals	Poland: Beskid Żywiecki Mts, Pilsko Mt., Hala Cebula (KRA 109402) Czech Republic: Moravia, Jesenik vill. (KRA 0205617)
Allium schoenoprasum L.	*Cepa/ Shoeneoprasum*	papyraceous to chartaceous	pattern of rectangular to elliptic cells	rectangular to elliptic cells with thick cell walls and calcium oxalate crystals	Poland: Skarżysko-Kamienna (KRA 0361634) Poland: Zła Wieś vill. near Świecie (KRA 0369366)
Allium atropurpureum Waldst. & Kit.	*Melanocrommyum/ Melanocrommyum*	membranous	striate	rectangular elongated cells with calcium oxalate crystals	Romania: Banatus, Timiș-Torontal (KRA 0205188) Romania: Temes, Vinga (KRA 459521)
Allium nigrum L.	*Melanocrommyum/ Melanocrommyum*	membranous to chartaceous	cobwebby	reticulate pattern	Italy: Sardinia, Su Nuraxi near Barumini (KRA 0386771)
Allium monanthum Maxim.	*Microscordum/ Microscordum*	papery	herringbone	herringbone pattern	Japan: Honshu (KRA 0311580)
Allium suaveolens Jacq.	*Polyprason/ Daghestanica*	papery	pattern of rectangular/ hexagonal cells	rectangular/ hexagonal cells with calcium oxalate crystals	Hungary: Veszprém vill. (KRAM 427744) Austria: near Vienna (KRA 079950)
Allium saxatile M.Bieb.	*Polyprason/ Oreioprason*	coriaceous	smooth	hexagonal cells with calcium oxalate crystals in the form of fine grains typically deposited by gravity on bottom wall of cell	Bosnia and Herzegovina: Trebinje (KRA 0278277) Azerbaijan: Quba (KRA 0205468)

TABLE 1. *Cont.*

Species	Subgenus/ section	Character of outer bulb tunic	Ultrasculpture of outer bulb tunic	Cell pattern of inner surface of outer bulb tunic	Herbarium data
Allium oreophilum C.A.Mey.	*Polyprason/ Polyprason*	papery	smooth	hexagonal to rectangular cells with calcium oxalate crystals	Uzbekistan: Tashkent distr. (KRA 0205438)
Allium moschatum L.	*Polyprason/ Scorodon*	fibrous	reticulate	elongated cells	Hungary: Budapest, Blockoberg Mt. (KRA 0205389, KRA 0205931)
Allium inconspicuum Vved.	*Reticulatobulbosa/ Campanulata*	fibrous	reticulate	elongated cells	Uzbekistan: Tashkent distr. (KRA 0205235, KRA 0205234)
Allium trachyscordum Vved.	*Reticulatobulbosa/ Flaviscordum*	fibrous	reticulate	elongated cells	KRA 0205305
Allium flavidum Ledeb.	*Reticulatobulbosa/ Reticulatobulbosa*	fibrous	reticulate	elongated cells	KRA 0205172
Allium lineare L.	*Reticulatobulbosa/ Reticulatobulbosa*	fibrous	reticulate	elongated cells	Ukraine: Lugansjkiensis (KRA 060756)
Allium splendens Willd. ex Schult.f.	*Reticulatobulbosa/ Reticulatobulbosa*	fibrous	reticulate	elongated cells	Russia: Kamchatka Peninsula (KRA 0205635)
Allium strictum Schrad.	*Reticulatobulbosa/ Reticulatobulbosa*	fibrous	reticulate	elongated cells	Ukraine: Podolian upland, Gołogóry Mts., Łysa (KRA 060742) Slovakia: Spišská Nová Ves (KRA 0205637)
Allium ochroleucum Waldst. & Kit.	*Rhizirideum/ Oreiprason*	fibrous	smooth to striate	rectangular elongated cells	Slovakia: Velká Fatra Mts, Drjenok (KRA 0205314) Austria: Kärnten, Kanalthal (KRA 0205317)
Allium albidum Fisch.	*Rhizirideum/ Rhizirideum*	membranous	striate	rectangular elongated cells with calcium oxalate crystals	Armenia: Sevan (KRA 062845) (KRA 0204937)
Allium angulosum L.	*Rhizirideum/ Rhizirideum*	membranous	striate	rectangular/ hexagonal elongated cells	Poland: Baranów vill. near Busko-Zdrój (KRA 0407262) Poland: Kotlina Sandomierska valley, Sieniawa vill. (KRA 0333962)
Allium flavescens Besser	*Rhizirideum/ Rhizirideum*	subcoriaceous	striate	rectangular/ hexagonal elongated cells	Romania: (KRA 0205140)
Allium senescens L.	*Rhizirideum/ Rhizirideum*	membranous	striate	rectangular/ hexagonal elongated cells	Hungary: Pest, Pilisszántó (KRA0205383) Slovakia: Prielom Hornádu (KRA 0205387)

ilarity of characters in taxonomically different species, and sometimes there is a great variation of ultrasculpture within subgenera. Such a case was reported for leaf epidermal pattern (Krahulec 1977, 1980). Some bulb tunic characters might also be modified by environmental conditions, and some structures may be

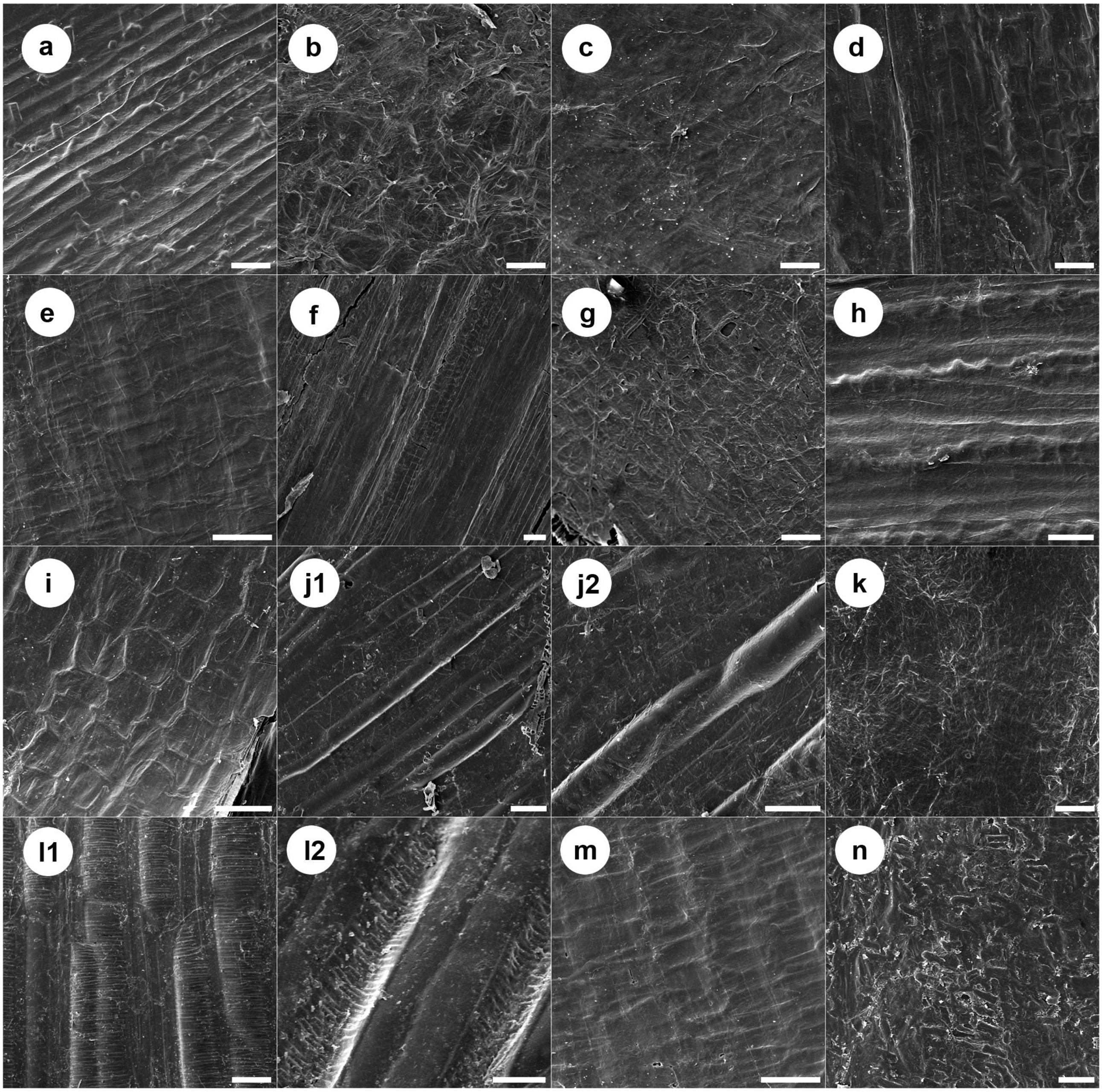

Fig. 1. Ultrasculpture of outer bulb tunic (inner surface) of examined *Allium* species. (**a**) *Allium acutiflorum*, (**b**) *A. ampeloprasum*, (**c**) *A. atroviolaceum*, (**d**) *A. polyanthum*, (**e**) *A. scorodoprasum* subsp. *rotundum*, (**f**) *A. scorodoprasum* subsp. *scorodoprasum*, (**g**) *A. sphaerocephalon*, (**h**) *A. vineale*, (**i**) *A. carinatum*, (**j1**, **j2**) *A. flavum*, (**k**) *A. melanantherum*, (**l1**, **l2**) *A. oleraceum*, (**m**) *A. paniculatum*, (**n**) *A. stamineum*. Bar = 50 μm.

adaptations to local habitat factors (Krahulec, 1980; McNeal and Ownbey, 1982; McNeal, 1992).

Following are findings for particular subgenera.

Allium subgenus *Allium*

The species referred to this subgenus were characterized by considerable variation. Bulb tunic ultrasculpture was striate with distinct ribs (Fig. 1a,h,j,l), hexagonal (Fig. 1e,g,i,m) or smooth to reticulate (Fig. 1b–d,k,n). Eight taxa of this subgenus had hexagonal cells with numerous or less numerous calcium oxalate crystals (Figs. 5a,d,e–g,i, 6a,b). The other species had elongated cells. Calcium oxalate crystals were fairly common in the bulb tunics of members of this subgenus (Tab. 1; compare with data on crystals in epidermal tissue in Mathew, 1996). Only *A. vineale* showed no crystals. The inner surface of the outer

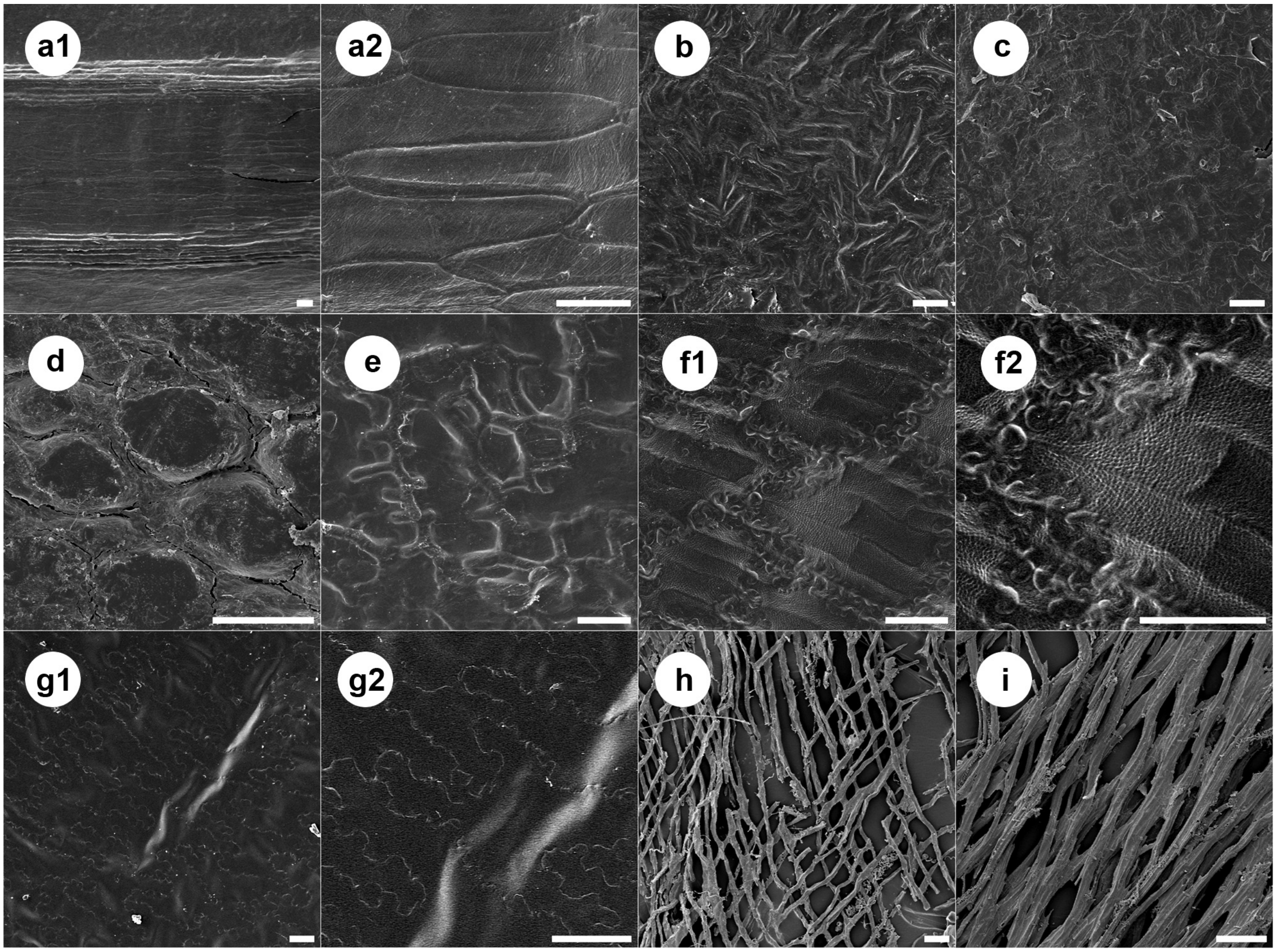

Fig. 2. Ultrasculpture of outer bulb tunic (inner surface) of examined *Allium* species. (**a1**, **a2**) *Allium ursinum*, (**b**) *A. pendulinum*, (**c**) *A. triquetrum*, (**d**) *A. chamaemoly*, (**e**) *A. moly*, (**f1**, **f2**) *A. roseum*, (**g1**, **g2**) *A. subhirsutum*, (**h**) *A. tricoccum*, (**i**) *A. victorialis*. Bar = 50 μm.

bulb tunic of *A. ampeloprasum* was composed of elongated cells with numerous oxalate crystals (Fig. 5b); in contrast, De Wilde-Duyfjes (1976) observed hexagonal cells in African specimens. My study showed subtle differences between the outer bulb tunics of *A. ampeloprasum* and *A. atroviolaceum*. The latter differs in having distinct reticulate fibrous outer tunics with prominent ribbing (Fig. 5c), which are absent in *A. ampeloprasum* (Fig. 5b) (see also descriptions in Vvedensky, 1935; Wendelbo, 1985). *Allium sphaerocephalon* showed two types of ultrasculpture and cell pattern depending on bulb tunic texture. In membranous bulb tunics, the cell pattern was hexagonal to rectangular with numerous oxalate crystals (Fig. 5g); more coriaceous bulb tunics had striate ultrasculpture with rectangular elongated cells. Similar observations were made by De Wilde-Duyfjes (1976), who presented three types of bulb tunic cell pattern. *Allium vineale* bulb tunics showed a characteristic ribbed structure with elongated cells, as also found by De Wilde-Duyfjes (1976) and McNeal and Jacobsen (2002), but high magnification also revealed papillary structure of the ribs (Fig. 1h). *Allium oleraceum* had distinct ultrasculpture with longitudinal ribs (compare with illustration in Aedo, 2013), with additional ultrasculptural details: ribs covered with spiral protrusions (Fig. 1l). The outer bulb tunic of *A. paniculatum* was membranous, with hexagonal cells (Fig. 6a), similar to the description from De Wilde-Duyfjes (1976), who reported, however, that the outermost bulb tunic leaf had longitudinal ribs, not observed here. *Allium stamineum* had outer bulb tunics without nerves, consistent with the description from Vvedensky (1935) but contradicting Wendelbo (1985); its numerous calcium oxalate crystals (Fig. 6b) gave it a specific ultrasculpture visible by SEM (Fig. 1n).

Allium subgenus *Amerallium*

This subgenus showed the most distinctive structures, with great diversity of outer bulb tunic ultrasculpture.

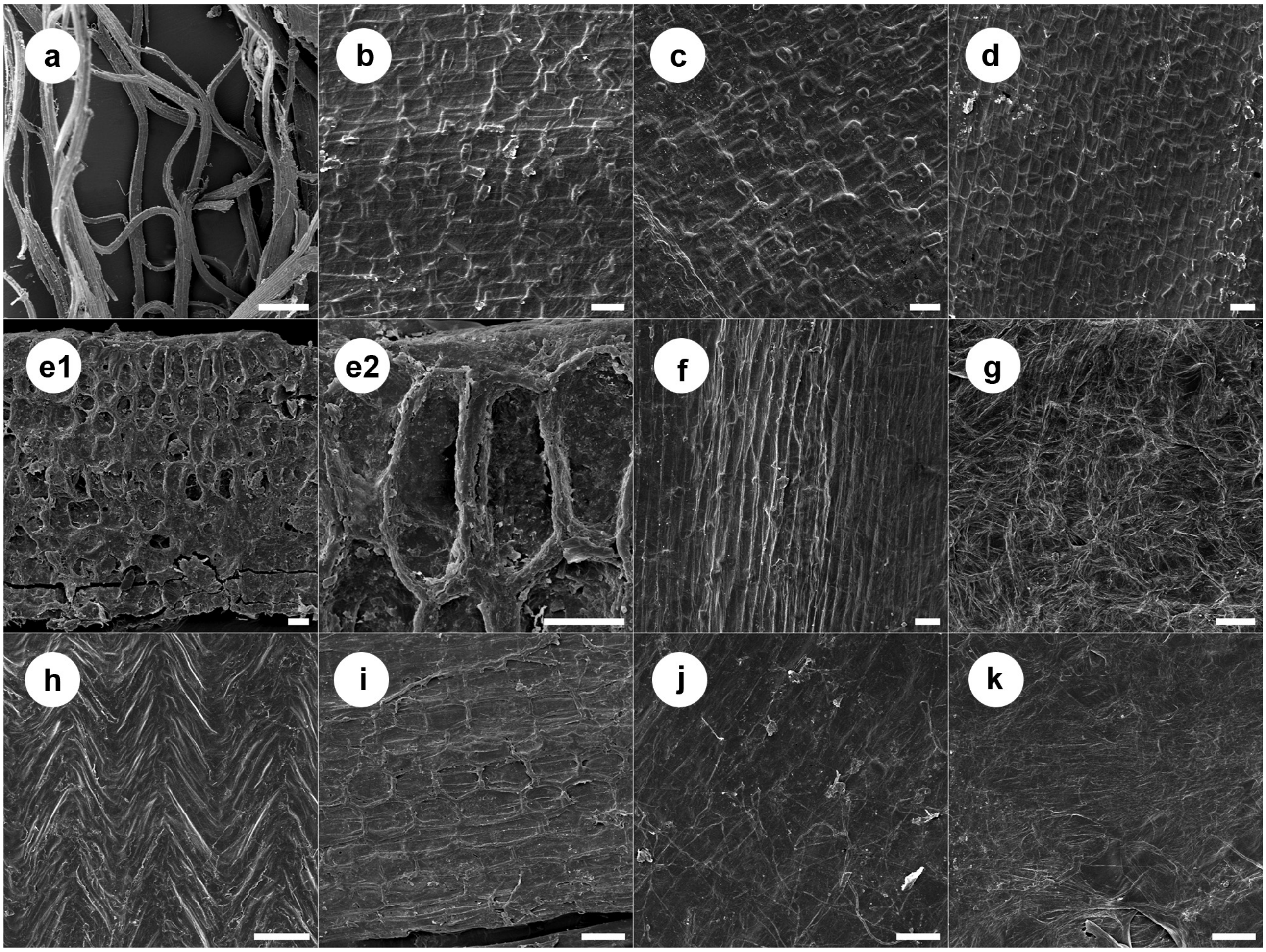

Fig. 3. Ultrasculpture of outer bulb tunic (inner surface) of examined *Allium* species. (**a**) *Allium ramosum*, (**b**) *A. cepa*, (**c**) *A. fistulosum*, (**d**) *A. sibiricum*, (**e-1**, **e-2**) *A. schoenoprasum*, (**f**) *A. atropurpureum*, (**g**) *A. nigrum*, (**h**) *A. monanthum*, (**i**) *A. suaveolens*, (**j**) *A. saxatile*, (**k**) *A. oreophilum*. Bar = 50 μm.

Species-specific differences were recorded and certain patterns were characteristic for particular species. The representative of section *Arctoprasum* – *A. ursinum* – showed a distinct type of cell pattern with elongated cells (Figs. 2a, 6c). A similar cell arrangement was observed in other members of subgenus *Amerallium* from North America, for example *A. lemmonii* S.Watson (McNeal, 1992) and *A. punctum* L.F. Hend. (McNeal and Jacobsen, 2002), but in those cases the ultrasculpture showed thick lignified cell walls (McNeal, 1992). *Allium pendulinum* had curved cells with sinuate cell walls (Figs. 2b, 6d), with ultrasculpture somewhat resembling that reported by McNeal (1992) for North American taxa of this subgenus: *A. tribracteatum* Torr. and *A. nevadense* S. Watson. *Allium triquetrum* did not have distinct ultrasculpture and its cell pattern consisted of roughly regular parallelograms (Fig. 6e). Similar observations were made by Aedo (2013). *Allium chamaemoly* had characteristic rhomboidal ultrasculpture (Fig. 2d) visible even at low magnification, as also reported by Aedo (2013). The pattern in *A. moly* was similar to *A. pendulinum* but the walls were much thicker (Figs. 2e, 6f). In *A. roseum* the inner layer of the bulb coat has been described as pitted and sclerified (Coste and Flahault, 1906; De Wilde-Duyfjes, 1976; Pastor and Valdés, 1983; Kollmann, 1984). Previous reports indicated finely sinuate cell walls (De Wilde-Duyfjes, 1976); in my study additional ultrasculptural details were visible (Fig. 2f). In fact the pitted structure of outer bulb tunics results from semilunar tubercular appendages clearly visible under SEM (Fig. 2f). All examined specimens of *A. subhirsutum* had sinuous cell walls in which no two cells appeared to have the same shape (Figs. 2g, 6g). Similar observations were made by De Wilde-Duyfjes (1976) but she found that three subspecies differ in the bulb tunic cell pattern; two of them, *A. subhirsutum* subsp. *subvillosum* and subsp. *spathaceum*, proved to have sinuous cells.

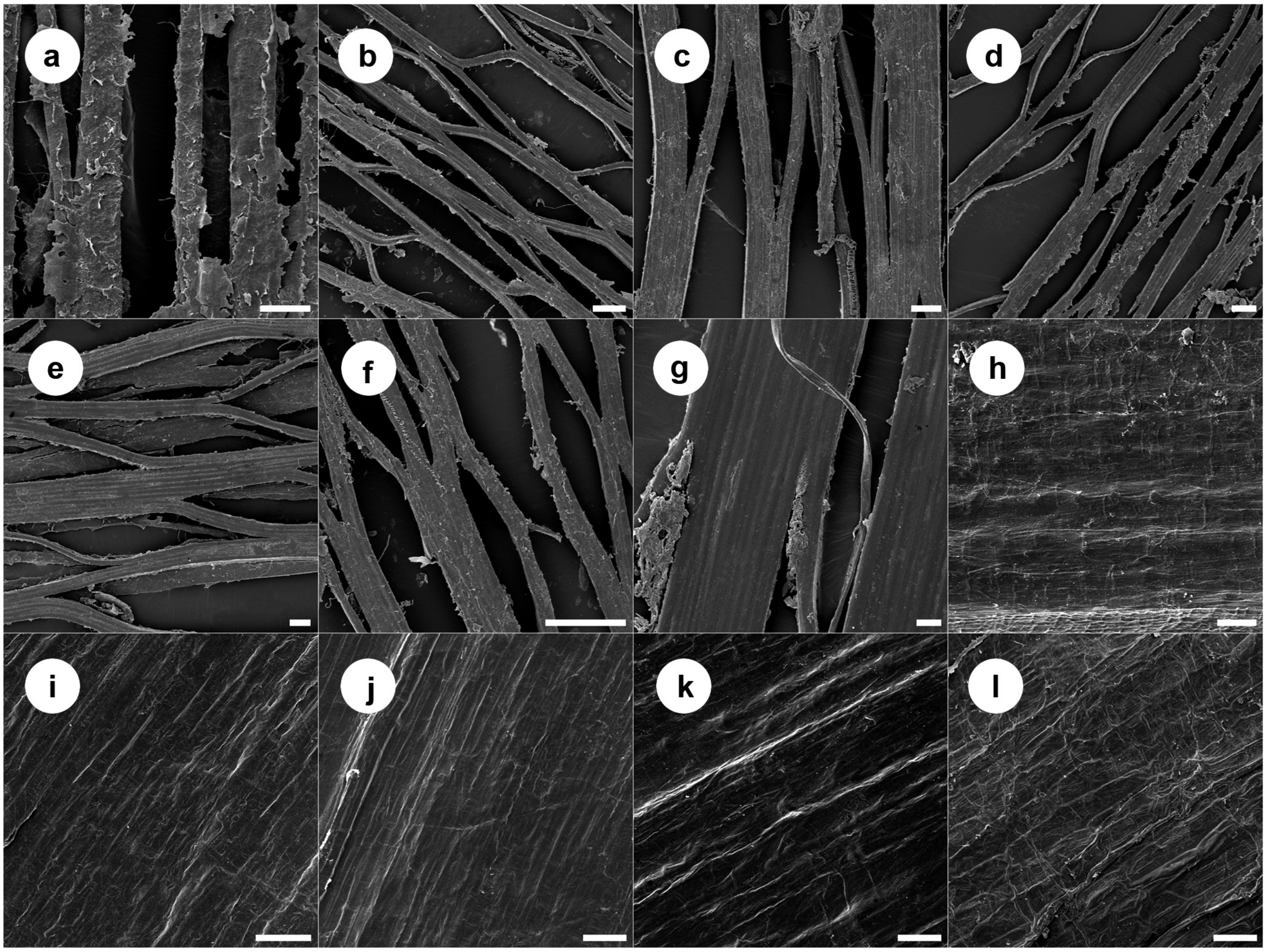

Fig. 4. Ultrasculpture of outer bulb tunic (inner surface) of examined *Allium* species. (**a**) *A. moschatum*, (**b**) *A. inconspicuum*, (**c**) *A. trachyscordum*, (**d**) *A. flavidum*, (**e**) *A. lineare*, (**f**) *A. splendens*, (**g**) *A. strictum*, (**h**) *A. ochroleucum*, (**i**) *A. albidum*, (**j**) *A. angulosum*, (**k**) *A. flavescens*, (**l**) *A. senescens*. Bar = 50 μm.

Species of this subgenus are distributed mainly in the Mediterranean area, but a number of species with similar characters occur in North America (Badr and Elkington, 1978). The bulb tunic ultrasculpture of North American representatives of subgenus *Amerallium* has been examined and deemed valuable in elucidating relationships among the North American species (McNeal, 1992). The native North American taxa of subgenus *Amerallium* were found to be a well-supported monophyletic clade, sister to the Old World taxa (Nguyen et al., 2008). Scape anatomical characters have also indicated close relationships between Old World and New World species of subgenus *Amerallium* (Fritsch, 1993). The species examined during my study showed great variation of bulb tunic ultrasculpture and certain patterns proved to be characteristic for particular species. The ultrasculpture pattern of North American representatives has also been found to be species-specific (McNeal and Ownbey, 1982; McNeal, 1992; McNeal and Jacobsen, 2002), while a special type of leaf anatomy, scape anatomical structures and hypodermal laticifers in bulb scales are constant and characteristic for the whole subgenus (Huang and Sterling, 1970; Fritsch, 1988, 1993).

Allium subgenus *Anguinum*

Allium victorialis and *A. tricoccum* were characterized by fibrous and reticulate tunics (Fig. 2h,i). The bulb tunic creates a specific reticulum structure consisting of elongated cells. This result confirms previously published reports (e.g., Pastor and Valdés, 1983; Xu and Kamelin, 2000; Aedo, 2013). A similar arrangement has been observed among other species from Korea and northeastern China representing the same sister group of species, belonging to subgenus *Anguinum*: *A. microdictyon* Prokh. and *A. ochotense* Prokh. (Choi and Oh, 2011).

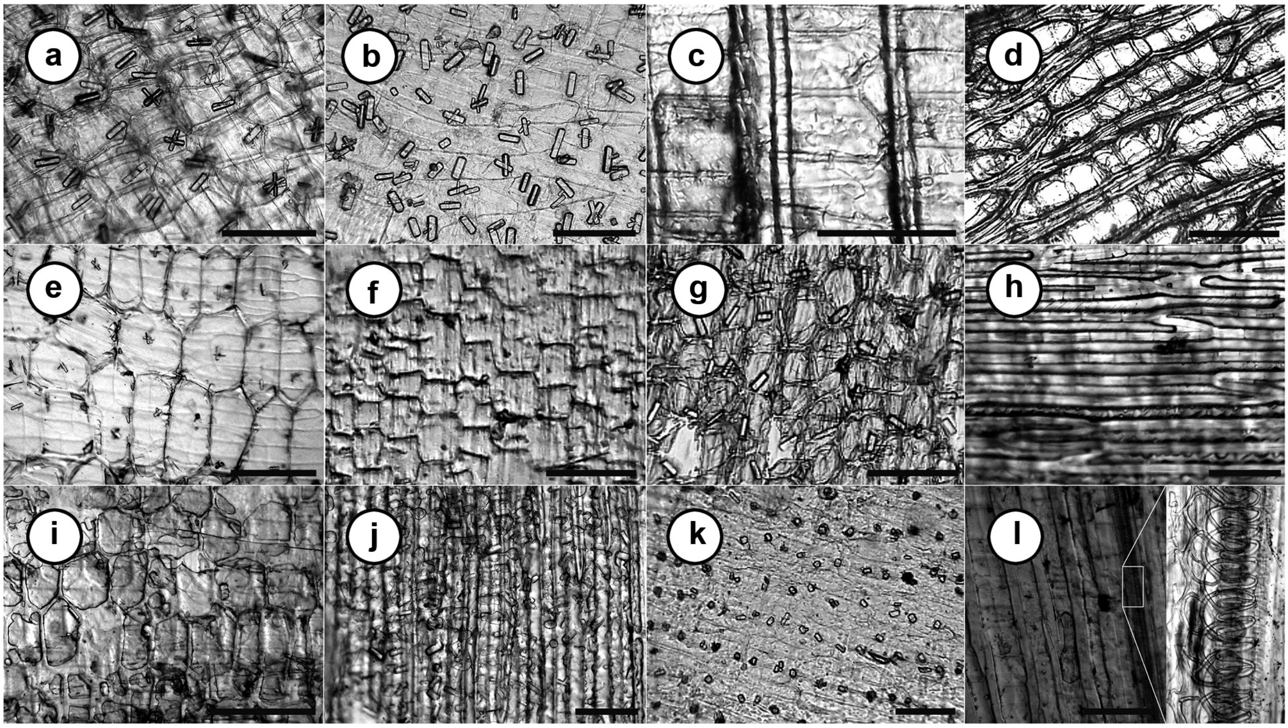

Fig. 5. Cell pattern of outer bulb tunic (inner surface) of selected *Allium* species. (**a**) *Allium acutiflorum*, (**b**) *A. ampeloprasum*, (**c**) *A. atroviolaceum*, (**d**) *A. polyanthum*, (**e**) *A. scorodoprasum* subsp. *rotundum*, (**f**) *A. scorodoprasum* subsp. *scorodoprasum*, (**g**) *A. sphaerocephalon*, (**h**) *A. vineale*, (**i**) *A. carinatum*, (**j**) *A. flavum*, (**k**) *A. melanantherum*, (**l**) *A. oleraceum*. Bar = 100 μm.

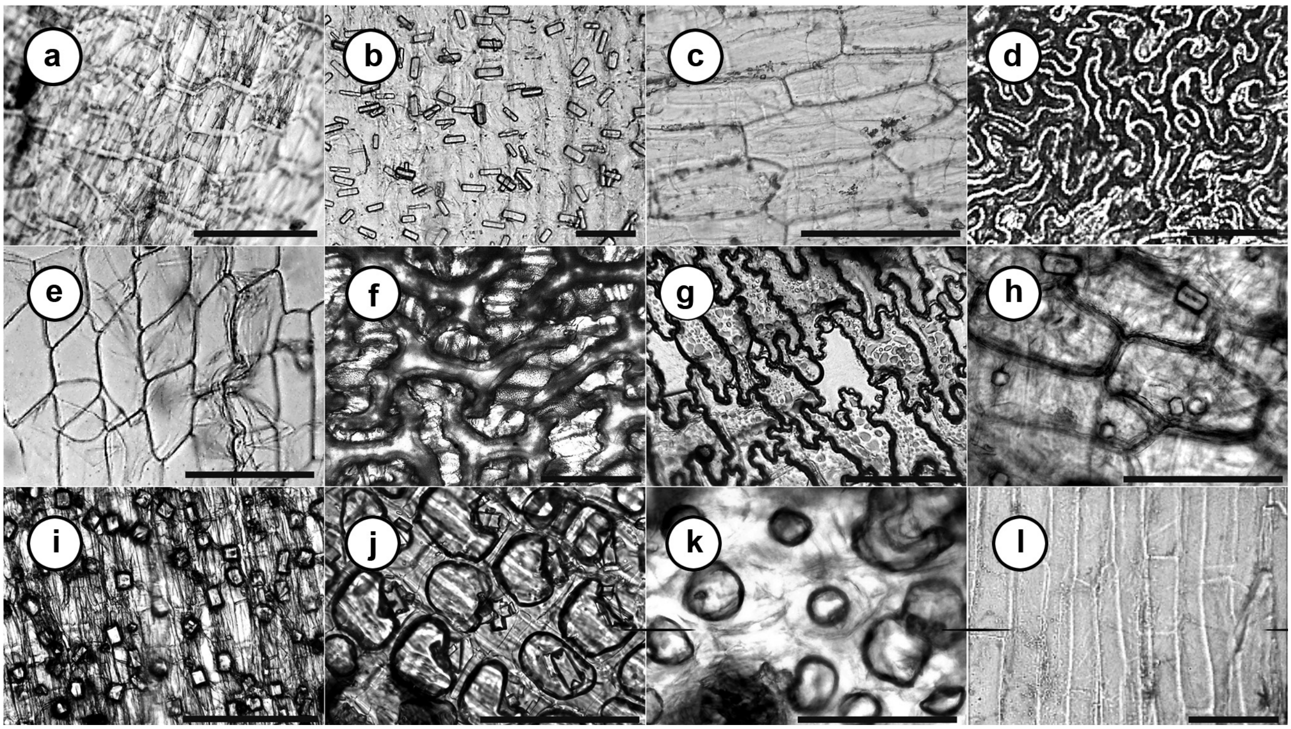

Fig. 6. Cell pattern of outer bulb tunic (inner surface) of selected *Allium* species. (**a**) *Allium paniculatum*, (**b**) *A. stamineum*, (**c**) *A. ursinum*, (**d**) *A. pendulinum*, (**e**) *A. triquetrum*, (**f**) *A. moly*, (**g**) *A. subhirsutum*, (**h**) *A. cepa*, (**i**) *A. fistulosum*, (**j**) *A. sibiricum*, (**k**) *A. schoenoprasum*, (**l**) *A. atropurpureum*. Bar = 100 μm.

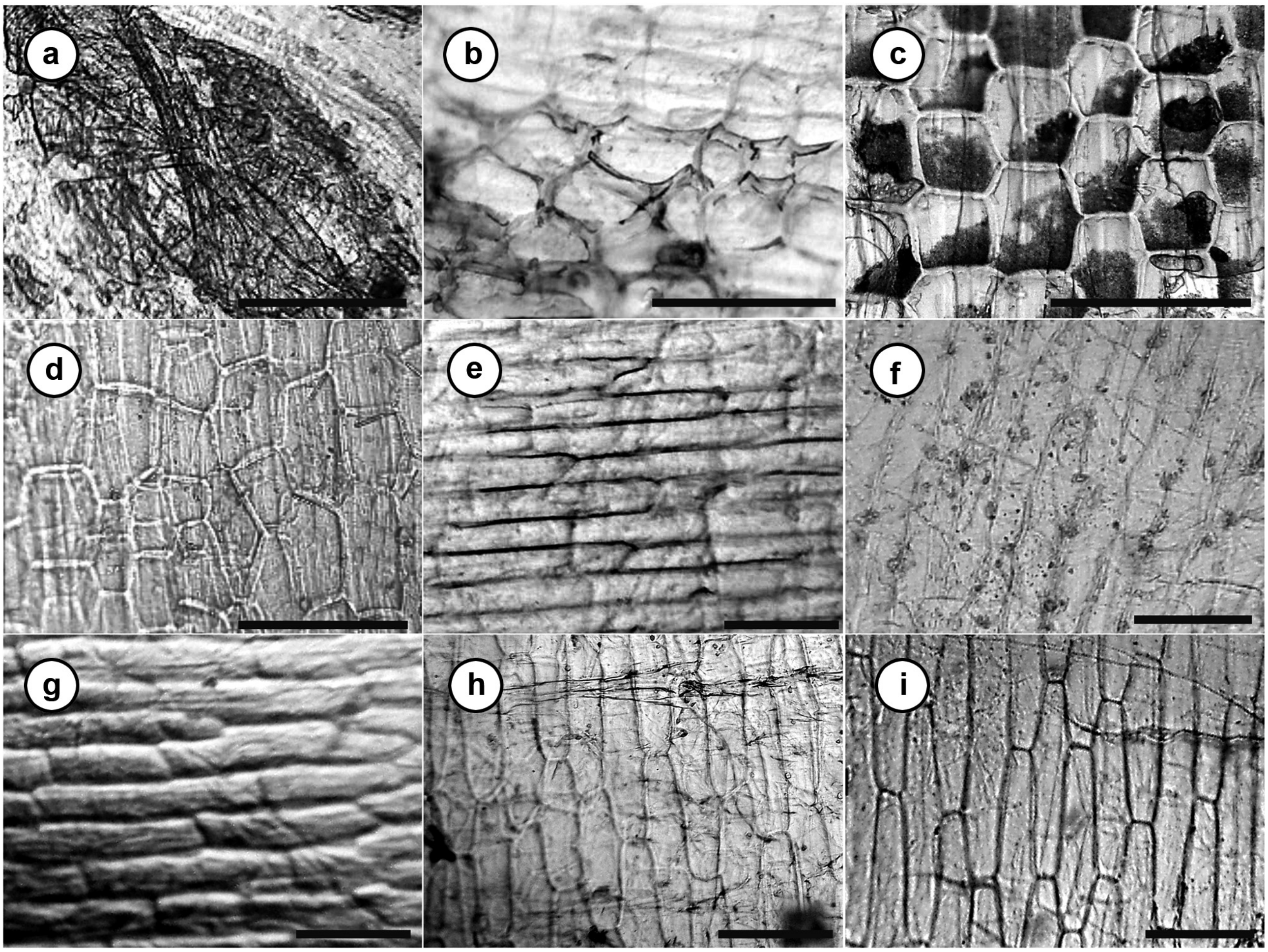

Fig. 7. Cell pattern of outer bulb tunic (inner surface) of selected *Allium* species. (**a**) *Allium nigrum*, (**b**) *A. suaveolens*, (**c**) *A. saxatile*, (**d**) *A. oreophilum*, (**e**) *A. ochroleucum*, (**f**) *A. albidum*, (**g**) *A. angulosum*, (**h**) *A. flavescens*, (**i**) *A. senescens*. Bar = 100 μm.

Allium subgenus *Butomissa*

Allium ramosum, the only representative of this subgenus examined in this study, is easily distinguished by its characteristic fibrous and reticulate tunics (Fig. 3a) consisting of elongated cells, consistent with results from Choi and Oh (2011).

Allium subgenus *Cepa*

A pattern of rectangular to elliptic cells was recorded (Fig. 6h–k). The sculpture was quite distinct and consisted of cells with thick walls, giving the bulb tunic an almost perforated structure (Figs. 3b–e, 6h–k). Calcium oxalate crystals were very common components of such ultrasculpture in this subgenus. The calcium oxalate was crystallized in the form of regular pyramidal prisms (Fig. 6h,i) or such prisms with blunt ends (Fig. 6j). Such crystals correspond respectively to the *cepa* and *schoenoprasum* types described by Chartschenko (1932).

Allium subgenus *Melanocrommyum*

No specific pattern was identified for this subgenus. *Allium atropurpureum* showed striate ultrasculpture with cells remarkably elongated (Figs. 3f, 6l). In contrast, *A. nigrum* develops a specific network on the bulb tunic surface (Figs. 3g, 7a) and consequently a specific ultrasculpture visible by SEM. Such structure has not been observed previously; Aedo (2013) reported striate ultrasculpture with elongated spindle-shaped cells.

Allium subgenus *Microscordum*

Allium monanthum showed a very distinct herringbone pattern of ultrasculpture (Fig. 3h), as also given by Choi and Oh (2011), but such a pattern cannot be regarded as specific to this subgenus. Some North American taxa of subgenus *Amerallium* (e.g., *A. crispum* Greene, *A. bolanderi* S. Watson, *A. serra* McNeal & Ownbey) also have such bulb tunic ultrasculpture (McNeal, 1992; McNeal and Jacobsen, 2002; Nguyen et al., 2008). In this context, note that subgenus *Microscordum* is most closely related to subgenus *Amerallium* (Friesen et al., 2006).

Allium subgenus *Polyprason*

The examined taxa did not present distinct ultrasculpture under SEM. Hexagonal to rectangular cell patterns were recorded (Fig. 7b–d). *Allium moschatum* was characterized by a bulb tunic which breaks into strips (Fig. 4a) and consequently it resembles reticulate ultrasculpture. *Allium saxatile* had characteristic hexagonal cells with calcium oxalate crystals in the form of fine crystal grains typically deposited by gravity on the bottom wall of the cell (Fig. 7c). This corresponds to the *globosum* type of calcium oxalate crystals described by Chartschenko (1932).

Allium subgenus *Reticulatobulbosa*

The bulb tunics are fibrous with reticulate ultrasculpture and a linear arrangement of cells (Fig. 4b–g). An almost similar appearance was described for species of the same section, *Reticulatobulbosa*: *A. koreanum* H.J.Choi & B.U.Oh and *A. splendens* Willd. ex Schult.f. (Choi and Oh, 2011).

Allium subgenus *Rhizirideum*

The taxa show characteristic linear ultrasculpture under SEM, following the long axis of the elongated cell walls (Figs. 4h–l, 7e–i). This pattern proved to be specific to this subgenus. Krahulec (1980) found that leaf epidermal anatomy is also uniform in this subgenus.

CONCLUSION

The ultrasculpture and cell pattern of the outer bulb tunic are generally differentiated within particular evolutionary lines of *Allium*. Although those characters do not directly indicate basal or advanced evolutionary levels, variation of ultrasculpture is sufficient to distinguish species within subgenus *Amerallium*, and characters of ultrasculpture or cell shape are diagnostic for some subgenera. The results indicate that *Allium* bulb tunic ultrasculpture is a useful character for taxonomic delimitation and species determination. It should help in analysis of the relationships between species of the first evolutionary line (Friesen et al., 2006), particularly in representatives of subgenus *Amerallium*.

ACKNOWLEDGEMENTS

I thank Prof. Zbigniew Szeląg (Jagiellonian University, Cracow) for valuable advice and for verifying specimen determinations, and Dr. Olga Woźnicka (Jagiellonian University, Cracow) for technical assistance.

REFERENCES

AEDO C. 2013. *Allium* L. In: Castroviejo S et al. [eds], *Flora Iberica*, vol. 20, http://www.rjb.csic.es/floraiberica/ (accessed 11.12.2013).

APG III. 2009. An update of the Angiosperm phylogeny group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* 161: 105–121.

BADR A, and ELKINGTON TT. 1978. Numerical taxonomy of species in *Allium* subgenus *Molium*. *New Phytologist* 81: 401–417.

CELEP F, KOYUNCU M, FRITSCH RM, KAHRAMAN A, and DOĐAN M. 2012. Taxonomic importance of seed morphology in *Allium* (Amaryllidaceae). *Systematic Botany* 37: 893–912.

ČEŠMEDŽIEV IV, and TERZIJSKI D. 1997. A scanning electron microscopic study of the spermoderm in *Allium* Subgen. *Codonoprasum*. Alliaceae. *Bocconea* 5: 755–758.

CHARTSCHENKO W. 1932. Verschiedene Typen des mechanischen Gewebes und der kristallinischen Ausbildungen als systematische Merkmale der Gattung *Allium*. *Beihefte zum Botanischen Centralblatt* 50: 183–206.

CHOI HJ, and COTA-SÁNCHEZ JH. 2010. A systematic review of *Allium* (Alliaceae) in the Canadian Prairie Provinces. *Botany/Botanique* 88: 787–809.

CHOI HJ, and OH BU. 2011. A partial revision of *Allium* (Amaryllidaceae) in Korea and north-eastern China. *Botanical Journal of the Linnean Society* 167: 153–211.

CHOI HJ, DAVIS AR, and COTA-SÁNCHEZ JH. 2011. Comparative floral structure of four New World *Allium* (Amaryllidaceae) Species. *Systematic Botany* 36: 870–882.

CHOI HJ, GIUSSANI LM, JANG CG, OH BU, and COTA-SÁNCHEZ JH. 2012. Systematics of disjunct northeastern Asian and northern North American *Allium* (Amaryllidaceae). *Botany* 90: 491–502.

CHOI HJ, JANG CG, KO SC, and OH BU. 2004. Leaf epidermal structure of the *Allium* L. and its taxonomic significance. *Korean Journal of Plant Taxonomy* 34: 97–118.

COSTE H, and FLAHAULT CH. 1906. *Flore Descriptive et Illustrée de la France*, vol. 3. Paris.

DE WILDE-DUYFJES BEE. 1976. A revision of the genus *Allium* L. (Liliaceae) in Africa. *Belmontia* 7: 75–78.

FAY MF, and CHASE MW. 1996. Resurrection of Themidaceae for the Brodiaea alliance, and recircumscription of Alliaceae, Amaryllidaceae and Agapanthoideae. *Taxon* 45: 441–451.

FRIESEN N. 1988. *Lukovye Sibiri: Sistematika, Kariologija, Khorologija*. Nauka-Sibirskoe Otd, Novosibirsk, Russia.

FRIESEN N, FRITSCH RM, and BLATTNER FR. 2006. Phylogeny and new intrageneric classification of *Allium* (Alliaceae) based on nuclear ribosomal DNA ITS sequences. *Aliso* 22: 372–395.

Fritsch RM. 1988. Anatomische Untersuchungen an der Blattspreite bei *Allium* L. (Alliaceae)-I. Arten mit einer einfachen Leitbündelreihe. *Flora, Morphologie, Geobotanik, Oekophysiologie* 181: 83–100.

Fritsch RM. 1992. Septal nectaries in the genus *Allium*. In: Hanelt P, Hammer K, Knupffer H [eds], *The Genus* Allium *– Taxonomic Problems and Genetic Resources*, 77–85. IPK, Gatersleben.

Fritsch RM. 1993. Anatomische Merkmale des Blütenschaftes in der Gattung *Allium* L. und ihre systematische Bedeutung. *Botanische Jahrbücher für Systematik* 115: 97–131.

Fritsch RM, and Abbasi M. 2009. New taxa and other contributions to the taxonomy of *Allium* L. (Alliaceae) in Iran. *Rostaniha* 10 Suppl. 1: 1–72.

Fritsch RM, Blattner FR, and Gurushidze M. 2010. New classification of *Allium* L. subg. *Melanocrommyum* (Webb & Berthel) Rouy (Alliaceae) based on molecular and morphological characters. *Phyton* 49: 145–220.

Fritsch RM, Kruse J, Adler K, and Rutten T. 2006. Testa sculptures in *Allium* L. subg. *Melanocrommyum* (Webb & Berth.) Rouy (Alliaceae). *Feddes Repertorium* 117: 250–263.

Garbari F, Greuter W, and Miceli P. 1979. The '*Allium cupanii*' group: a preliminary taxonomic, caryological and leaf anatomical study. *Webbia* 34: 459–480.

Güler U, and Pehlivan S. 2006. Pollen morphology of some species belonging to *Codonoprasum* and *Allium* sections of *Allium* (Liliaceae, Alliaceae). *Biologia* 61: 449–455.

Gurushidze M, Fritsch RM, and Blattner FR. 2008. Phylogenetic analysis of *Allium* subgen. *Melanocrommyum* infers cryptic species and demands a new sectional classification. *Molecular Phylogenetics and Evolution* 49: 997–1007.

Huang SM, and Sterling C. 1970. Laticifers in the bulb scales of *Allium*. *American Journal of Botany* 57: 1000–1003.

Jaccard P, and Frey A. 1928. Kristallhabitus und Ausbildungsformen des Calciumoxalat als Artmerkmal. *Festschrift Hans Schinz. Beiblatt zur Vierteljahresschr. Naturf. Ges. Zürich* 73 (15): 127–161.

Kollmann F. 1984. *Allium* L. In: Davis PH. [ed.], *Flora of Turkey and the East Aegean Islands*, vol. 8, 98–210. Edinburgh University Press, Edinburgh.

Krahulec F. 1977. Poznámky k určování československých česneků (*Allium*) v nekvetoucím stav. *Zprávy České Botanické Společnosti* 12: 145–159.

Krahulec F. 1980. Epidermal characters of *Allium* species autochthonous in Czechoslovakia: their pattern, taxonomic and ecological relationships. *Preslia* 52: 299–309.

Kruse J. 1984. Rasterelektronenmikroskopische untersuchungen an samen der gattung *Allium* L. *Kulturpflanze* 32: 89–101.

Kruse J. 1992. Variability of testa sculptures in the genus *Allium* L. In: Hanelt P, Hammer K, Knupffer H [eds], *The Genus Allium – Taxonomic Problems and Genetic Resources*, 181–182. IPK, Gatersleben.

Mathew B. 1996. *A Review of Allium Section Allium.* Royal Botanic Gardens, Kew.

McNeal DW. 1982. Taxonomy of the *Allium lacunosum* complex (Liliaceae). *Madroño* 29: 79–86.

McNeal DW. 1992. Taxonomy of North American species of *Allium*. In: Hanelt P, Hammer K, Knupffer H [eds.], *The Genus* Allium *– Taxonomic Problems and Genetic Resources*, 195–204. IPK, Gatersleben.

McNeal DW, and Jacobsen TD. 2002. *Allium*. In: *Flora of North America*, *Flora of North America north of Mexico*, vol. 26, 224–276. Oxford University Press, New York.

McNeal DW, and Ownbey M. 1973. Bulb morphology in some western North American species of *Allium*. *Madroño* 22: 10–24.

McNeal DW, and Ownbey M. 1982. Taxonomy of the *Allium lacunosum* complex (Liliaceae). *Madroño* 29: 79–86.

Namin HH, Mehrvarz SS, Zarre S, and Fritsch RM. 2009. Pollen morphology of selected species of *Allium* (Alliaceae) distributed in Iran. *Nordic Journal of Botany* 27: 54–60.

Neshati F, and Fritsch RM. 2009. Seed characters and testa sculptures of some Iranian *Allium* L. species (Alliaceae). *Feddes Repertorium* 120: 322–332.

Nguyen NH, Driscoll HE, and Specht CD. 2008. A molecular phylogeny of the wild onions (*Allium*; Alliaceae) with a focus on the western North American center of diversity. *Molecular Phylogenetics and Evolution* 47: 1157–1172.

Pastor J. 1981. Contribución al estudio de las semillas de las especies de *Allium* de la Península Ibérica e Islas Baleares. *Lagascalia* 10: 207–216.

Pastor J, and Valdés B. 1983. Revisión del género *Allium* (Liliaceae) en la peninsula Ibérica e Islas Baleares. PhD Disseration, Universidad de Sevilla, Sevilla.

Pastor J, and Valdés B. 1986. Bulb structure in some species of *Allium* (Liliaceae) of Iberian Peninsula. *Annales Musei Goulandris* 7: 249–261.

Prychid CJ, and Rudall PJ. 1999. Calcium oxalate crystals in monocotyledons: a review of their structure and systematics. *Annals of Botany* 84: 725–739.

Rola K. 2012. Taxonomy and distribution of *Allium ursinum* (Liliaceae) in Poland and adjacent countries. *Biologia* 67: 1080–1087.

Speta F. 1984. Über Oberösterreichs wildwachsende Laucharten (*Allium* L., Alliaceae). *Linzer Biologische Beitraege* 16: 45–81.

Stearn WT. 1978. European species of *Allium* and allied genera of Alliaceae: a synonymic enumeration. *Annales Musei Goulandris* 4: 83–198.

Stearn WT. 1980. *Allium* L. In: Tutin TG, Heywood VH, Burges NA, et al [eds], *Flora Europaea*, vol. 5, 49–69. Cambridge University Press, Cambridge.

Vvedensky AI. 1935. Rod 267. Luk- *Allium* L. In: Komarov VL. [ed.], *Flora URSS*, vol. 4, 112–280. Izd. Akad. Nauk SSSR, Leningrad.

Wendelbo P. 1985. *Allium* L. In: Townsend CC, and Guest E [eds], *Flora of Iraq*, vol. 8, 137–177. Ministry of Agriculture and Agrarian Reform, Baghdad.

Xu JM, and Kamelin RV. 2000. *Allium* L. In: Wu ZY, Raven PH. [eds], *Flora of China*, vol. 24, 165–202. Science Press, Beijing; Missouri Botanical Garden Press, St. Louis.